PROCESS FOR DEPOSITING STRONG ADHEREND POLYMER COATING ONTO AN ELECTRICALLY CONDUCTIVE SURFACE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention concerns a process for depositing by electro-grafting a polymer coating onto an electrically conductive surface.

2. Description of the Prior Art

It is well known that the surface of a variety of organic and inorganic substrates is coated by synthetic polymers in order to impart them specific properties (e.g. adhesion, hydrophilicity or hydrophobicity, low friction, resistance to environmental attack, biocompatibility, etc.). These coated surfaces are an extreme example of composites, comprising constitutive components having a layered structure. In such a field, a major problem to be solved is the usually weak and short-term adhesion between completely different substrates, such as metal, glass, carbon and organic polymer. Covalent bonding at the interface is often considered as a target to be reached. In this respect, electropolymerisation of acrylates or methacrylates has proved to be a powerful tool to deposit polymers strongly adhering to electrically conducting substrates. Delamar and al in Carbon 1997,35,801 have demonstrated that electroreduction of these acrylates or methacrylates monomers at an appropriate potential, leads to a rapid formation of a homogeneous polymer coating on the cathode whatever its shape (plate, fiber) and nature (metals alloys, carbon, Indium Tin Oxideglass,...).

However, several problems limit the use of electropolymerization to practical applications: film thickness is small (<100 nm) due to a fast termination of grafted polymer brushes, and the monomers which can be electrografted are restricted to one (although large) family of activated vinylic monomers e.g.acrylate, cyano, cyano-acrylate, pyridine... compounds. As a rule, the carbon-carbon double bond must

be activated by an electron withdrawing substituent for the monomer to be reactive at the cathode and to be adsorbed on it preferably to the solvent. Additionaly, any function, such as alcohol, protic amine and carboxylic acid, which are reduced at a less cathodic potential than the monomer cannot be tolerated.

These serious restrictions considerably shorten the list of eligible monomers, hence the list of strongly adherent coatings that can be achieved on conductive surface via electro-grafting, and hence the usefulness of electrografted coatings in practical applications.

In particular when the surface of the substrate belongs to metallic prostheses such as for example structure-support implants, as for example bones plates, bone screws, femoral heads, stent, dental implants problems of biostability and biocompatibility are important.

For example, when applied to stent which serves as scaffolding to the vessel wall after percutaneous vessel enlargement, a well known limitation to existing stents is their limited hemo and tissue- compatibility. After stent implantation, inflammatory response does occur which in turn might promote local thrombosis activation and/or smooth muscle cells and myofibroblasts migration and proliferation. Stent implantation might also delay normal endothelial regeneration after vessel injury. This might ultimately results in further lumen obstruction impeding normal blood flow to the heart. This process has been called restenosis. Therefore, there is a need to develop more biocompatible stent possibly capable to release bioactive compounds to modify tissue reactions to metallic stent implantation. Previous attempts to coat metallic stents with polymers have failed in part due to inadequate bonding between the polymer matrix and the stent surface. Indeed, with previous coating techniques, stent deployment and/or sterilization created breaks, fissures in the polymers or detachment of the coating from the stent surface. In addition, preliminary results on the release of drugs inhibiting restenosis, such as sirolimus or taxol, seem to indicate that adequate global average release rates amount to a 100 to a few 100's of micrograms of drug over a few months. Alternatively, it is known that the release kinetics remains controlled by the encapsulating polymer as far as the drug content in the polymer matrix remains lower

than about 10 to 20%. This also indicates the need for a better adhesion and an increase thickness of drug releasing polymer layers for this type of application.

A certain number of applications require barrier coatings, capable either to prevent corrosion of the underlying metal (cars, medical devices such as stems, dental implants, guidewires etc), or to disfavor adsorption of biological molecules onto the surface when put into contact with biological fluids and/or implanted. Such problems is encountered e.g. in biochips (non specific adsorption, which affects signal-to-noise detection ratios), and in general on the active zone of physical or chemical sensors (e.g. adsorption of proteins on pressure sensors, glucose sensors etc), as well as for packaging applications on micro-systems (should they be implanted or not). In these cases, polymers stemming from electro-graftable monomers often fail to provide a good a durable protective coating, either because of their structure, or because of their low thickness, or both. Additionally, very low thickness is also synonymous with a high sensitivity to scratching: the thickness of traditional electro-grafted polymers is by far lower than the rugosity of the surface of most objects, which thus pit the electro-grafted layer as soon as they are put into contact with it, ruining its tentative protective characteristics.

Intermediate thicknesses (i.e. of the order of a micron or of a few microns) are also desirable to get insulating layers capable of resisting mechanical contact, insertion/desinsertion or friction cycles, such as in connectic (computers, mobile phones...etc), micromechanics and electrical devices in general in which a metallic part is put into contact with the polymer coating. As a rule, the rugosity of the metallic part is higher than one micrometer (since getting to lower rugosities requies specific treatments), and durable coatings must therefore be thicker than one micron.

A clear need thus emerges for strongly adhering polymer coatings on conductive surfaces about a micrometer or even a few micrometers thick.

One definite advantage of the electro-grafting is the capability to force the formation of interface - presumably covalent - carbon/metal bonds, with high grafting ratios: parameters of the electrochemical protocol can be defined to favour the formation

of grafted polymer brushes, for which controlling the thickness amounts to controlling the molecular mass of the grafted chains. As outlined above, the growth of polymer chains is probably hindered and stopped at an early stage, leading to pretty short chains, or in any case to chains the length of which cannot be easily controlled on the basis of traditional macromolecular strategies. A first direction towards obtaining thicker polymer films is thus to be able to achieve the grafting of polymer chains of high molecular mass.

Besides the physical characteristics of the polymer layer (thickness, compacity...etc), the very chemical nature of the polymer layer to be grafted is also important.

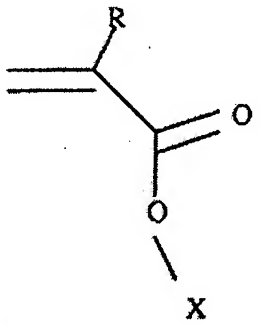
Microsystems designed for biomedical applications are packaged by biocompatible coatings of polymeric nature, such as e.g. parylene (which is deposited by Chemical Vapor Deposition, CVD) or PDMS (Poly DiMethyl Siloxane), which enable a correct biocompatibility as well as an interesting chemical resistance. High performance lubrication capabilities - i.e. low friction coefficients - are offered by perfluoro polyether layers, e.g. on connectors or mechanical parts. None of these polymers can be obtained as the result of electro-initiated propagation reaction. Identically, one cannot straightforwardly get e.g. electro- grafted poly-imides, poly-amides (which are good candidates in microelectronics for highly insulating polymers as they afford low k dielectric layers), and in general polymers obtained by polycondensation reactions. Rather than thinking of which vinylic polymer may provide properties identical to these materials, the need is great to provide a process thanks to which these polymers may nevertheless be grafted onto conducting surfaces.

Due to the relatively short list of functional groups an activated vinylic monomer can bear to be eligible for electrografting, electro-grafted polymers offer a restricted catalog of functionalities to fix additional layers by conventional chemical methods, such as those used e. g. in the solid support synthesis of peptides, the fixing of olinucleotides (DNA chips) or proteins (protein chips). Thus, even in the restricted domain of activated vinylic monomers, a need remains to enlarge the list of molecules capable of providing reactive coatings by electro-grafting.

SUMMARY OF THE INVENTION

We have now found a process for depositing by electro-grafting new types of strong adherent polymer coating onto an electrically conductive surface, which solve the problems mentioned above.

Accordingly, the present invention provides a process for depositing by electro-grafting a strong adherent polymer coating on an electrically conductive surface comprising an electrochemical grafting at the surface of an active monomer for forming a primer coating P onto said surface and having as general formula:



wherein R represents hydrogen or methyl
and the monomer comprises an X group
which is part of a preformed polymer or
is an intermediate agent for polyaddition reaction or
is an anchoring group for attachment of a molecule having at least one complementary reactive group.

When X is part of a preformed polymer, the monomer becomes a macromonomer bearing at least one activated vinylic pendant group, e.g. an acrylic or methacrylic function. Such approach allows formation of new primer by one-step electro-grafting of a reactive polymer called macromonomer.

Such process also allows further modification of an initial electrografted polymer coating (called primer coating P) to increase the coating thickness by the so-called

grafting-from technique i.e. polymerization of a second monomer (called M in Fig.2, see below) or to introduce other types of polymers (also called top coating) via covalent attachment between the primer and the top coating through the X ester group by the so-called grafting-onto technique.

Such process also allows to graft onto the primer coating compounds like functional polymer, peptide, protein, oligonucleotide, dyes, drugs, anti bacterian compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically illustrates electrografting of a monomer on a conductive surface S with a solvent A and a conducting salt B to form a primer electrografted polymer coating P.

Figure 2(I) schematically illustrates formation of a top-coating (TC) by a grafting-from technique i.e. polymerization of a second monomer M from the group X of an electrografted polymer which serves as grafted macroinitiator or transfer agent.

Figure 2(II) schematically illustrates formation of a top-coating (TC) by a grafting-onto technique i.e. introduction of other types of preformed polymers or any reactive molecule or macromolecule (O-Y) via covalent attachment between the primer P and the top coating TC through the X ester group of an electrografted monomer.

Figure 3 schematically illustrates electrografting of macromonomers ((1)-(2) or (3)).

Figure 4 exhibits a voltammogram showing passivation of a cathode by polyethyl acrylate coating (PEA).

Figure 5 exhibits a voltammogram for electrografting of 1-acryloxy-2-phenyl-2-(2',2',6',6'-tetramethyl-1'-piperidinyloxy)ethane (ATEMPO) (a) first scan (b) second scan on the same cathode.

Figure 6 exhibits a plot of Raman signal intensity at $1000cm^{-1}$ versus grafted polystyrene molecular Mass Mn measured on samples prepared by initation of styrene by

nitroxide mediated radical polymerization (NMP) from the poly(ATEMPO) electrografted coating for various amounts of free alkoxyamine in solution.

Figure 7 exhibits a volammogram for electrografting of poly(2-chloropropionate ethyl acrylate. (a) first scan (b) second scan illustrates passivation of the cathode by the resulting electrografted polymer coating.

Figure 8 exhibits a plot of Raman signal intensity at $1000cm^{-1}$ versus a grafted polystyrene molecular Mass Mn measured on samples prepared by initation of styrene by atom transfer radical polymerization (ATRP) in presence of various amounts of benzyl bromide.

Figure 9 exhibits a voltammogram for electrografting of a macromonomer: poly(4-acryloyloxy-ε-caprolactone-co-ε-caprolactone) (a) first scan (b) second scan showing passivation of the cathode by the resulting electrografted polymer coating.

Figure 10 exhibits a cyclic voltammograms at various scanning rates for ferrocene amine grafted-onto polyacrylate succinimide (grafting-onto technique).

Figure 11 exhibits UV-VIS spectra recorded for biotine-cadaverine grafted onto polyacrylate succinimide (a) after formation of a complex with dimethylaminocynnamaldehyde (b) before complex formation.

Figure 12 illustrates release of Rhodamine-6G from apoly-ε-caprolactone coating.

Scheme 1 illustrates a three-step preparation pathway for a poly(ε-caprolactone) coating by ring opening polymerization of ε-caprolactone from electrografted polyethylacrylate.

Scheme 2 illustrates electrografting of 1-acryloxy-2-phenyl-2-(2',2',6',6'-tetramethyl-1'-piperidinyloxy)ethane (1) and subsequent grafting from of polystyrene by nitroxide mediated radical polymerization (2).

Scheme 3 illustrates electrografting of poly(2-chloropropionate ethyl acrylate) and the subsequent grafting from of polystyrene by atom transfer radical polymerization.

Scheme 4 illustrates various structures of macromonomers based on poly(ε-caprolactone) backbone and suitable for direct electrografting.

Scheme 5 illustrates structure of a macromonomer based on poly(ethylene glycol) backbone used for directelectrografting.

Scheme 6 illustrates electrografting of N-acryloyloxysuccinimide (a) and a subsequent grafting onto of polystyrene containing primary amine groups (b).

DESCRIPTION OF THE INVENTION

By electrografting, and as illustrated in Fig. 1, wherein A is a solvent and B a conducting salt, one means the electrografting of the monomer on the conductive surface S to form a primer electrografted polymer coating P. In such electrochemical process, the monomer is electrochemically polymerized and simultaneously the so-formed polymer is electrografted onto S.

By macromonomer, one means, a preformed polymer bearing at least one acrylic or methacrylic function as illustrated in Fig. 3.

By grafting-from, and as illustrated in Fig.. 2(I), one means, initiation of the polymerization of a second monomer M from the group X of the grafted monomer which serves as grafted macroinitiator or transfer agent.

By grafting onto, and as illustrated in Fig.. 2(II), one means bonding of O-Y, a preformed polymer or any reactive molecule or macromolecule onto the conductive surface, through the Y complementary reactive group with the X group of the monomer.

By intermediate agent one means an initiator or a transfert agent for a polyaddition reaction.

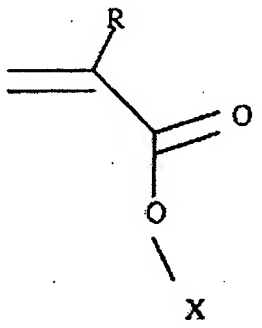
The conducting surface according to the invention is for example steel, stainless steel, Inox316L, tantalum, titanium, nitinol, carbon, ITO glass, transition metal (Fe, Ni, Cu. Au, Ag,...), metal doped polymer.

The electrochemical grafting at the surface is performed under well known conditions from a solution of the monomer in an aprotic solvent A containing a conducting salt B. The monomer solution is subjected to electrolysis so as to create a

reaction for example a cathodic reaction wherein the conductive surface to be coated is used as cathode at a potential situated in the range of the electron transfer between the conductive surface and the monomer of a value equal or near the value corresponding to an inhibition peak of the cathodic reaction of the monomer, but less negative than the second reduction peak corresponding to the polymerization in solution and degrafting of the polymer coating.

A first approach to solve the problems mentioned above is to combine the electro-grafting technique with a polymerization reaction.

In its first aspect, the present invention provides a process for depositing by electro-grafting a polymer coating on an electrically conductive surface comprising an electrochemical grafting at the surface of an active monomer for forming a primer coating P onto said surface and having as general formula:



wherein R represents hydrogen or a methyl

and the X group is an intermediate agent for polyaddition reaction.

The monomer is bearing an intermediate agent of polymerization in the ester group X and is electrografted onto the solid surface.

The intermediate agent may be an initiator for ring opening polymerization (ROP), for polymerization via nitroxyde radicals (NMP), for atom transfer polymerization (ATRP) or a transfert agent for polymerization via reversible-addition-fragmentation (RAFT).

This type of approach is particularly interesting as it allows a global covalent grafting of a variety of polymers onto conductive surfaces which is much larger than the

restricted list of polymers whose monomers is strictly eligible for electro-grafting. The later list is the one dictated by the aforementioned constraints of cathodic electro-grafting, namely the absence of labile protons, and the activation of the vinylic double bond by electron withdrawing groups. For example, this new type of approach allows coatings of polymers other than polyacrylates or polymethacrylates. Such new approach allows coatings of polymers such as for example polystyrene or polyhydroxy ethylacrylate to be deposited on the conducting substrates with a strong adhesion and an increased and tunable thickness.

The polyaddition according to the invention may be controlled or not. It may be a ring opening polymerization (ROP)[as described by P; Dubois et al. in; Makromol. Chem., Macromol. Symp. 42/43, 1991, 103], a radical polymerization such as for example atom transfer polymerization (ATRP) [as described in Matyjaszewski, Curr. Org. Chem., 2002, 6, 67], polymerization via nitroxyde radicals (NMP) [as described in Chem. Rev., 2001, 101, 3661], polymerization via reversible-addition-fragmentation (RAFT) [as described by Moad et al., in polym. Int., 49, 993, 2000] or a combination of two different controlled polyaddition such as ATRP and ROP.

The Ring opening polymerization (ROP) may be applied to lactones and lactides such as (E-caprolactone), and functional caprolactones such as y- bromo E-caprolactone, or lactide such as D,L-Lactide...or any other polymerizable cyclic monomer such as trimethylene carbonate, cyclic anhydride, glycolide....

In ROP, X may be a metal carboxylate such as aluminium carboxylate or a metal alkoxide such as aluminium alkoxide, stanneous alkoxide, titanium alkoxide, magnesium alkoxide or zinc alkoxide.

Monomers used as precursor for ROP process are for example trimethylsilylhydroxy(meth)acrylate, glycidyl (meth)acrylate, ethyl acrylate ROP experimental conditions are well known by the man skilled in the art.

The radical polymerization may be applied for the obtention of vinyl polymer of a wide range and predetermined molecular weight.

Examples of radical polymerization are ATRP, AMP, RAFT.

In NMP, an alkoxyamine is used as initiator and mediator: A C-O bond of the alkoxylamine is thermally homolytically cleaved into two free radicals: a carbon centered radical, to initiate the polymerization and a nitroxide radical to regulate the radical polymerization. The cleavage of the C-O bond occurs by heating at a temperature between 40°C and 160°C, preferably 110°C.

In ATRP, X may be a haloalkane, haloketone, haloester, halonitrile, haloalkylbenzene or sulfonyl chloride or any other function able to initiate atom transfer radical polymerization. For this process, an additional metallic complex has to be added with the monomer to catalyze the polymerization. The metal of such catalyst has to be chosen to be compatible with the surface. On easily oxidizable surfaces like iron, a commonly used cupper catalyst has to be replaced by a more stable ruthenium based catalyst.

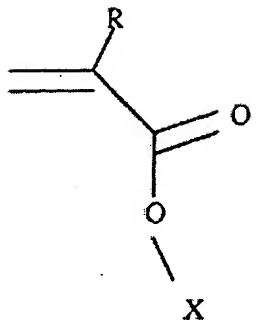
The controlled polymerization via reversible-addition-fragmentation (RAFT) may be applied to obtain vinyl polymer as described by Lee and al in WO9801478 Al. In RAFT, X may be for example a dithioester, a dithiocarbamate, a trithiocarbonates or any other function able to control reversible-addition-fragmentation polymerization.

All polyaddition are performed under general conditions well known by the man skilled in the art.

A combination of the electro-grafting technique with a polymerization reaction as mentioned in the first aspect of the present invention allows a controllable increase in the thickness of the coating at the surface and a better adherence of the polymer coating.

A second approach to solve the above problems is to use a macromonomer instead of a monomer.

In its second aspect, the present invention provides a process for depositing by electro-grafting a polymer coating on an electrically conductive surface comprising an electrochemical grafting at the surface of an active monomer for forming a primer coating onto said surface and having as general formula:



wherein R represents hydrogen or methyl
and X is part of a preformed polymer.

In such approach, the monomer becomes a macromonomer bearing at least one acrylic or methacrylic function to be electrografted on the surface S as illustrated in Figure 3.

The preformed polymer may be obtained by any type of polymerization technique such as by a controlled/living polymerization or not. The preformed polymer may be obtained for example by a polyaddition process with anionic, cationic, coordinative or radical initiation or by a polycondensation process.

The macromonomer may be an α- or an α-, ω-acrylic or methacrylic substituted polymer, a randomly acrylic or methacrylic functionalized copolymer, a diblock copolymer with one block bearing the acrylic or methacrylic groups, or any kind of macromolecular architecture (like stars, graft, tapered copolymers) which comprise active acrylic or methacrylic groups. Fig. 3 illustrates different examples of macromonomers. Macromonomer (1) is a polymer with pendent acrylic or methacrylic groups, Macromonomer (2) is a α functionalized polymer and (3) is an α-ω functionnalized polymer.

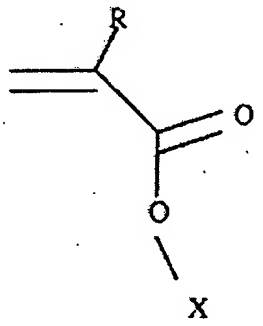
The macromonomer has to be compatible with the electrode polarization, so that no other electroactive functions in the range of electrografting potential, except the acrylic or methacrylic groups, is present on the macromolecular architecture. For instance, no unprotected alcohol, no carboxylic acid, no amine, no bromide should be present along the polymeric chain.

The macromonomer has also to be soluble in the electrochemical bath i.e. in polar solvents like dimethylformamide, dimethylsulfoxide, pyridine, acetonitrile, hexamethylphosphoramide,...

The process of electro-grafting with a macromonomer as mentioned in the second aspect of the present invention considerably broadens the list of organic polymer coatings which may be obtained straightforwardly by electro-grafting, enabling a chemical engineering of the coating at the monomer level. It also allows for a better adhesion of the polymer coating at the surface.

A third approach to solve the above problems is to combine electro-grafting with a grafting onto technique.

In its third aspect, the present invention provides a process for depositing by electro-grafting a polymeric coating on an electrically conductive surface comprising an electrochemical grafting at the surface of an active monomer for forming a primer coatingP onto said surface and having as general formula:



wherein R represents hydrogen or methyl

and X is an anchoring group for attachment of a molecule having at least one complementary reactive group.

In such approach, one have an X activated ester function towards nucleophilic compounds.

X may be for example a succinimidyl group particularly reactive towards amines. It may also be an epoxy, a vinyl, an allyl, an aryl, a chloride group or a combination of them.

Electro-grafting of such monomer provides a surface modified with a thin organic primer P suitable for further "grafting onto" process of a very wide range of molecules or macromolecules forming a new top coating (TC) onto the primer coating P.as illustrated in Fig. 2(II).

The main requirement for the "grafting onto" step is the presence of at least one complementary reactive group on the molecule or macromolecule to be grafted onto the precoated surface P. Therefore molecules or macromolecules to be used according to such aspect of the invention may be of various architecture obtained either by polycondensation or polyaddition reaction and bearing at least one complementary reactive group. They may be (for instance aminopolystyrene, aminopolyimide, aminopolydimethylsiloxane,...), proteins, enzymes, oligonucleotides, drugs, dyes, or small organic molecules of particular interest like electroactive molecules (aminoferrocene), vitamine (biotine), ligands,...

The combination of the electrografting technique with a grafting onto process of a very wide range of molecules or macromolecules as mentioned in the third aspect of the present invention is also advantageous to increase the coating thickness and to obtain a strong adhesion of the top coating.

Such approach is particularly helpful in medical applications to introduce biological polymers such as proteins which biocompatibilize and biostabilize the coated surface in its biological environment.

One further advantage of all three approaches is that the coated polymer may be used to entrap or immobilize a biologically active molecule (D). When these coatings are designed to be biocompatible and to play a biological function either intrinsically or by serving as a reservoir for biologically active molecules, they allow said molecules to play a biological function by local release or contact with adjacent tissues. The polymer coating is then able to withstand mechanical stress and sterilization process. In the particular case of coronary stems, the polymer coating is able to withstand deformation such as that induced by the inflation of the balloon for optimal stent deployment.

Particularly, said biologically active molecules are chosen for their ability to improve stent biocompatibility. Said active molecules might prevent, limit or suppress

neointima formation, thrombosis or inflammatory response after stent implantation. Alternatively said active molecules might accelerate normal endothelial cell regeneration after stent implantation. Such agents must have clear antiproliferative or antithrombotic or anticoagulant or endothelial-growth promoting capabilities. One such agent is a beta, alpha or gamma isotope which is complexed with an chelating agent and which might be incorporated into the proposed coating. Upon stent placement against vessel wall, said active molecules are slowly released and may interfere with the tissue reaction towards the metallic stent surface. The chelating agent may be hydrophilic or hydrophobic. Examples of chelating agents are ethylene diaminetetraacetic acid (EDTA), diethylene triaminepentaacetic acid (DTPA) and its analogues N-[2 amino-3-(rho-nitrophenyl)propyl]-trans- cyclohexane- 1,2-diamine-N,N',N" pentaacetic acid (nitro-CHX-A-DTPA) or 2-methyl-6-(rho-nitrobenzyl)-1,4,7 triazaheptane-N,N,N',N",N"- pentaacetic acid (nitro-lB4M-DTPA or nitro-MX DTPA) or deferoxamine (DFO) and derivatives, hydroxyethyl starch-conjugated deferoxamine (HES-DFO), 4 aminobenzylderivativetriethlenetetraaminohexaacetic acid (TTHA), 1,1 bis[(11 N-hydroxy)-2,5,11-triaza-1,610-trioxo dodecanyl] ethane (KD), ((+)-3-hydroxy 1-(2-hydroxyethyl)-2-hydroxyphenyl-methyl-1H-pyridin-4-one) (CGP 65015), sodium diethyldithiocarbamate(NaDDC), 2-(rho-nitrobenzyl)-1,4,7,10tetraazacycyclododecane-N',N",N"'-tetraacetic acid (nitro-DOTA), alpha-(2(rho-nitrophenyl)ethyl)-1,4,7,10-tetraazacyclododecane-1-acetic-4,7,10-tris(methylacetic) acid (nitro-PADOTA), 2-(rho-nitrobenzyl)-1,4,7,10 tetraazacyclotridecane-N,N',N",N"'-tetraacetic acid (nitro-TRITA), 6,6" bis[[N,N,N",N"- tetra(carboxymethyl)amino] methyl] 4'-(3-amino-4methoxyphenyl)-2,2':6',2"-terpyridine (TMT-amine), analogues of pyridoxal isonicotinoyl hydrazone (PIH), desferrithiocin (DFT), cysteine, O-phenantroline, 2-hydroxy-4- methoxypyridine-1-oxide, maltol, 1,2- Dimethyl-3 hydroxypyrid-4-one, sar, diamsar, 3-cholesteryl 6-[N'- iminobis(ethylenetrilo) tetraacetic acid]hexyl ether (Chol-DTTA), N,N'- Bis(3,4,5 trimethoxybenzyl) ethylediamine-N,N'-diacetic acid, salicylaldehyde isonicotinoylhydrazone (SIH), neocuproine, nitrilo triacetic acid (NTA), 8-hydryquinoline (8HQ), phosphatidyllethanolamine- diethylenetriaminetetraacetic acid (PE-DTTA), diplamitoyl-phosphatidyl-ethanolamine-monomethoxy polyethylene glycol

5000 (PE-MPEG), docosyl-triethylenetetraminepentaacetic acid (C22TT), 1,10-phenatroline (OP). Chelating agents may also serve as intracellular or extracellular therapeutic agent and interefere with specific biological functions. For example, such chelator may have antioxidant properties or anti- proliferative properties.

This type of encapsulation is also useful to design coatings capable of releasing locally proteins and oligonucleotides, and in general any molecule or macromolecule which may play a part in any biological reaction coming into play in the physiological acceptance of the object on the surface of which the polymer layer has been grafted.

Another advantage of the present invention in its three approaches is that any miscible polymers may be blended with the coating polymer from a solution in a solvent for both polymers by solvent casting or spin coating. Such blending is an additional way to increase the coating thickness.

The following examples are illustrating the invention. The spectral conditions are the same for all examples except where otherwise mentioned.

EXAMPLE 1

Poly(ε-caprolactone) coating by ring-opening polymerization of ε-caprolactone from Polyethylacrylate (hereafter called PEA) electrodeposited on stainless steel

Scheme 1

The process was tested on several metallic devices like steel plate, Be® stent (in stainless steel 316L), and Wiktor® stent (in tantalum). Electropolymerization onto the metallic device was performed as follows. The metallic device was washed with heptane and acetone, and dried overnight under vacuum. Residual oxides were electrochemically reduced in acetonitrile (Aldrich) /tetraethylammonium perchlorate (Merck) prior to use. The metallic device was immersed in a solution containing ethyl acrylate as monomer (EA, 1M, Acros), tetraethylammonium perchlorate (TEAP, 0.05 M) in dimethylformamide (DMF, Aldrich). Two platinum anodes and a platinum foil used as pseudo-reference were immersed in the solution, and the curves were recorded with an EG&G potentiostat/galvanostat (M273A). The potential applied to the metallic device

serving as a cathode was scanned down at a rate of 20 mV/s, and held constant at the value of the first reduction wave (**Fig. 4**, E=-2.2 V/Pt v = 20mV/s, AE 1M in DMF + TEAP: a) first scan, b) second scan). It was held at this value until the current is decreased due to passivation of the metallic device, by the poly(ethyl acrylate) coating (PEA). This passivation was confirmed by the absence of reduction during a second potential scanning. The PEA-coated metallic device was washed repeatedly with DMF and acetonitrile. The thin PEA coating having a thickness inferior to 100nm was characterized by IR-RAS spectroscopy. IR-RAS spectra were recorded by a reflexion-absorption technique (Brucker spectrophotometer) directly onto the modified electrodes whose surface reflects light. IR-RAS spectra exhibit the C=O ester band at 1739 cm^{-1}.

This first step is described in scheme 1, step 1.

The modified electrode with PEA (P in Figure 1) was dried by repeated azeotropic distillation of toluene. A volume of 1 ml of diisobutyl aluminum hydride (DiBAlH) (Aldrich) in hexane (1 10^{-3} mol/l) was added dropwise to the PEA-coated metallic device immersed in toluene, and was allowed to react for 16 hours. As a result ethyl ester was transformed into aluminum alkoxide (Scheme 1, step 2), initiator of the ring opening polymerization at RT of E caprolactone.(Aldrich) The reduction of ethyl ester was confirmed by IR-RAS spectroscopy. (IR-RAS spectra were recorded by a reflexion-absorption technique under air (Brucker spectrophotometer) directly onto the surface modified light- reflecting steel electrodes; the intensity of the transmitted signal being dependent on the film thickness). Since the aluminum alkoxide are unstable in the air, the reduced PEA is washed with non dried toluene which promoted the hydrolysis of alkoxide groups into alcohol for easy spectral characterization purpose. The large band at 3380 cm^{-1} is characteristic of alcohol groups which means that the alkoxide was previously formed. The band of ester carbonyl bond at 1739 cm^{-1} has decreased in intensity after a reduction time of 16 hrs, and has disappeared after 24 hrs. which underlines the bonding of the initiator.

Prior to the ring opening polymerization of ε-caprolactone (Aldrich), the metallic device was washed thoroughly in toluene under nitrogen in order to remove unreacted

DiBAIH. It was placed in a solution of 1 ml of ε-caprolactone (M in Figure 2) (9 10^{-3} mol) in 40 ml of toluene under stirring at room temperature (Scheme 1, step 3). Polymerization of ε-caprolactone was stopped after 12 hrs until 4 days by addition of an excess of HCl. The metallic device was washed extensively in toluene (Soxhlet extractor 4 days to 1 week) in order to remove the unreacted monomer and non grafted polymer if any, and then dried under vacuum. The poly(ε-caprolactone) coating (PCL) was confirmed by IR-RAS. The characteristic band of carbonyl groups of the PCL backbone was observed at 1728 cm^{-1}. The intensity of the signal depends on the polymerization time meaning that the polymer thickness increases with the polymerization time.

Thicknesses above some μm have been reached. The Raman spectrum also confirmed the coating of the metallic device by PCL. Raman diffusion spectroscopy was carried out with a Dilor spectrometer (SuperLabram type), equipped with a 800-2000 CCD detector cooled by liquid nitrogen and with a microscope. The spectral resolution was 2 cm^{-1}. The excitation laser beam was focused on the sample, the probed surface area being ca. 1μm^2 (100X lens Contact angles were measured by the sessile drop technique. 10 μl droplets of distilled water were deposited with a microsyringe onto the polymer surface, and static contact angles were measured. All the reported data were the average of ten measurements collected from different areas of the polymer surface. It was lower on the metallic plate coated with PEA-PCL than on PEA. The measured angle (78°) onto the metallic plate coated with PEA-PCL is lower than onto PEA (92°) and higher than onto reduced PEA (56°) and was similar to the film obtained by solvent casting of PCL on stainless steel which demonstrates that PCL is completely coating the underlying reduced PEA. Peeling measurements were carried out according to the ASTM standards D3330M-90. An adhesive tape (Acrylic foam 4930, 3M) was let to adhere on the polymer coating for 24 h. It was then peeled off, and the tensile strength between the tape and the PCL film surface was measured by an Instron tensile tester. An adhesion strength higher than 1070N/m was obtained (adhesion strength of the tape onto the top-coating) and the PCL top coating remains attached onto the metallic device after peeling. In comparison, the adhesion strength values of PCL cast films from chloroform solution was 830 N/m when PCL is solvent cast onto reduced PEA electropolymerized on steel and 70

N/m when PCL is solvent cast onto PEA electropolymerized on steel. In these both cases the PCL top coating was completely removed from the surface.

EXAMPLE 2

Poly(D,L-lactide) (hereafter called PLA) coating by ring-opening polymerization of D,L-lactide from PEA electrodeposited on steel

Following the same procedure as in **EXAMPLE 1**, but replacing ε-caprolactone (M in Figure 2) by D,L lactide, ring-opening polymerization of D,L lactide (Aldrich, 1.3 g, 0.4 M in toluene) was initiated by the aluminum alkoxide group of PEA electrodeposited on steel and reduced by DiBAIH. Polymerization occurred at 70°C in 40 ml of toluene, and was stopped after 72 hours by addition of an excess of HCI. The PLA coating was confirmed by Raman spectroscopy with the characteristic bands appearing at 1450 and 1750cm^{-1}. Similar thickness and adhesion strength as in case of PCL(example 1) have been obtained on these samples.

EXAMPLE 3

Poly(γ-bromo-ε-caprolactone) coating by ring-opening copolymerization of a mixture of γ-bromo-ε-caprolactone and ε-caprolactone from PEA electrodeposited on steel

Following the same procedure as in **EXAMPLE 1**, the ring-opening polymerization of pure γ-bromo-ε-caprolactone or in mixture with ε caprolactone (50:50 mol/mol, 1. 3 g, total concentration = 0.25, 0.5 or 0.75 M in toluene) was initiated by the aluminum alkoxide group (initiator previously called X in Figs. 1 & 2) of PEA electrodeposited on steel and reduced by DiBAIH. (pure y-bromo-E- caprolactone is prepared as described in M.Mazza and al in Macromolecules 2000, 33, 14).

Polymerization occurred at 70°C in 40 ml of toluene, and was stopped after a period of 72 hrs to 4 days by addition of an excess of HCI. The presence of bromine atoms in the coating was confirmed by X-ray fluorescence spectra wherein an intense signal characteristic of the Kα and Kβ peaks of the bromine atom are clearly seen. Again,

the thickness is increased up to some microns for high polymerization time (4 days). Adhesion strength of the tape to the top coating is in the range of the ones measured for pure polycaprolactone (see example 1), the film being also not removed after peeling.

EXAMPLE 4

Electrografting of 1-acryloxy-2 phenyl-2-(2',2',6',6'-tetramethyl- 1' piperidinyloxy) ethane and subsequent initiation of styrene by nitroxide mediated radical polymerization (NMP)

Scheme 2

a) Electrografting of 1-acrylaxy-2-phenyl-2-(2',2',6',6'-tetramethyl-1' piperidinylexy)ethane.

A steel or a nickel plate was immersed in a dry DMF solution of 1-acryloxy- 2 phenyl-2-(2',2',6',6'-tetramethyl-1'-piperidinyloxy)ethane (monomer hereafter called ATEMPO where X is a group bearing a nitroxyde type radical). The monomer is electrografted in the same experimental conditions as example 1, but ethylacrylate is replaced by the ATEMPO monomer.

Figure 5a shows the voltammogram characteristic for the electrochemical grafting of ATEMPO (0.4M) by scanning the potential to E=-1.8 V/Pt in a solution of TEAP (0.05M) in DMF, -. A decrease in the current intensity (curve b: second scan) is the signature of the electrode passivation by the formation of a polymer; coating.. X-ray photoelectron spectroscopy was performed on such samples (XPS was performed under ultra high vacuum (UHV), with a VG - ESCALAB I 20 220iXL spectrometer and the monochromatised Al Ka radiation at 1486.6 eV.

The sensitivity factor for each element was considered for quantitative analysis. Carbon, oxygen and nitrogen atoms were detected by XPS analysis of the surface. Their atomic ratios extracted from the XPS spectrum are C/O = 7. 6 and O/N = 3.75 which is in accordance with the theoretical value C/O = 6.7 and O/N = 3. Detection of signals from the underlying metallic substrate indicates that the grafted film is thinner than 10 nm. This first step is illustrated in scheme 2, step 1.

b) Initiation of styrene polymerization from the electrografted coating. scheme 2 step 2

The steel or the nickel plate coated by poly[1-acryloxy-2- phenyl-2- (2',2',6',6'-tetramethyl-1'-piperidinyloxy)ethane] was immersed in distilled styrene (Aldrich, 10 ml) (Monomer M in Figure 2) and the temperature was increased to 120°C for 24 h. Ungrafted polystyrene was removed by Soxhlet extraction with toluene for two weeks. The polystyrene coating was confirmed by Raman spectroscopy, with particularly intense bands at 1001, 1601,3053 cm^{-1}. The molecular weight and so the thickness of the polymer coating may be controlled by addition of free alkoxyamine (benzoate of 2- phenyl-2-(2',2', 6',6'-tetramethyl-1'-piperidinyloxy)ethyl up to 0.25mmol for 5ml of styrene) to the medium as illustrated in Figure 6. Indeed, the PS chains are attached to the poly[1-acryloxy-2-phenyl-2-(2',2',6',6'-tetramethyl-1'-piperidinyloxy)ethane] electrografted primer (called P in Figure 2) by an ester bond, which can be hydrolyzed at high pH (NaOH 10M in THF at room temperature during 3h to 24h). It is thus possible to release PS and to have it characterized by size exclusion chromatography (SEC measurements were carried in THF at 40°C using a Hewlett-Packard 1090 liquid chromatograph equipped with a 1037A refractive index detector (columns HP PL gel 5µ (10^5Å, 10^4 Å, 10^3 Å, 100 Å)) and a Waters 600 liquid chromatograph equipped with a 410 refractive index detector (columns styragel HR (HR1: 100-5000, HR2: 500- 20000, HR4: 5000-600000)).; The columns were calibrated with PS standards) . Because the probing depth of the Raman spectroscopy exceeds the thickness of the organic film, the intensity of the Raman signal is proportional to the film thickness. This expectation is confirmed by Figure 6, which shows that the intensity of the Raman signal for the aromatic units of PS at 1000 cm^{-1} linearly changes with molecular Mass Mn of the grafted PS chains. All the experimental conditions are similar to the ones described above. Power of laser, aperture, number of scans... remain constant. When no free alkoxyamine is added to styrene, the control of the chain growth is lost meaning that the polydispersity of the chains becomes higher (above 2) but still thick films (below 5µm thickness) of adhering polystyrene are obtained.

EXAMPLE 5: Electrografting of poly(2-chloropropionate ethyl acrylate I 30 with subsequent initiation of styrene (ATRP).

Scheme 3

A steel or a carbon plate was treated as example 1 but EA is replaced by 2-chloropropionate ethyl acrylate with a concentration of 0. 15M (chloropropionate is X as initiator) (Fig.7, E=-1.8 V/Pt, in DMF + TEAP: a) first scan, b) second scan). A decrease in the current intensity was the signature of the electrode passivation by the formation of a polymer coating. The chemical composition obtained by X-ray photoelectron spectroscopy is nearly in accordance with the theoretical values (C = 58. 3%, 0 = 28.4%, Cl = 8.3%): C = 62%, 0 = 31%, Cl = 7 %. Only a small amount of Cl atoms is missing due to the partial reduction of C-Cl bond during the electrografting step. This step is illustrated in scheme 3 step 1.

b) Initiation of styrene polymerization from the electrografted coating

The steel or the carbon plate coated by poly(cPEA) or by poly(cPEA-co-EA) was immersed in a solution of styrene and ATRP catalyst in toluene. For steel plates, the Grubbs catalyst ($RuCl2(=CHPh)(PCy_3)_2$) has been used while cupper catalyst with hexamethyltriethylenetetraamine (HMTETA) as ligand can be used for modification of the carbon substrates. The temperature was increased to 110°C for various period of time (4-24h). Ungrafted polystyrene was removed by Soxhlet extraction with toluene for two weeks. The polystyrene coating was confirmed by Raman spectroscopy. The molecular weight and so the thickness of the polymer coating can be control by addition of free initiator to the medium As for example 4, the molecular weight and so the thickness of the polymer coating may be controlled by addition of free initiator (benzyl bromide) to the medium as confirmed by Raman and SEC (Fig. 8). The thickness of the films reaches range between 1 to 5 m without addition of free initiator. Peeling test (ASTM D3330M-90) confirmed the deposition of an adherent PS coating. The adhesion strength is above 2340N/m. PS remains on the surface after peeling. This step is illustrated in scheme 3 step 2.

EXAMPLE 6

Poly (ε-caprolactone) coating by electrografting of a copolymer of ε-caprolactone and 4-acryloylaxy-ε-caprolactone onto steel.

Macromolecular approach. Scheme 4.

Poly(4-acryloyloxy-ε-caprolactone) (poly(ACL)) and poly(4-acryloyloxy-ε caprolactone-co-ε-caprolactone) (poly(ACL-co-CL)) are linear polyesters bearing pendant acryloyl groups along the polymer backbone called here macromonomers. Poly(ACL) with Mn = 12000 and Mw/Mn = 1.25 and poly(ACL-co- CL) with Mn = 15000, Mw/Mn = 1.20, and ACL/CL 41:59 (mol/mol) were synthesized by ring-opening polymerization with aluminum triisopropoxide as the initiator. The macromonomer is synthetised as described by X. Lou and al in Langmuir 2002, 18, 2785.

The steel device was treated as example 1 but EA is replaced by poly(ACL) or poly(ACL-co-CL) - with the concentration in acrylate groups of 1M Electrografting has been performed by scanning the potential (v:20mV/s) to the top of the first reduction peak (E: -1.8V/Pt, Figure 9, curve a) in the bath containing the macromonomer (1M in acrylate functions in Figure 9), all the other conditions being similar to example 1. The passivation is clearly evidenced by the very low current during the second scan (Figure 9 curve b). The polymer coated device was washed repeatedly with DMF and acetone. Contact angle of water, IR-RAS (with the bands at 2945 and 1743 cm^{-1} for alkyl and carbonyl absorption band), and peeling test (ASTM D3330M-90) confirmed the deposition of an adherent PCL coating. The adhesion strength is above 2800N/m for PACL and above 370N/m for PACL-co-CL. Scanning electron microscopy observations so as IR- RAS measurements indicated that poly(ACL-co-CL) coatings were thicker than poly(ACL) coatings which are around 350nm when prepared at high concentration.

EXAMPLE 7

Poly(ethylene glycol) network by electrografting dimethacrylate diethylene I glycol (DMADEG) onto steel.

Macromolecular approach. Scheme 5.

In the same conditions as example 1, a steel plate was immersed in a solution containing dimethacrylate diethylene glycol (DMADEG, with a concentration = 0.2 to 1M, Polyscience) (here called macromonomer) rather than ethyl acrylate. The first reduction wave was already observed at a DMADEG concentration of 0.2 M. The coating formed at a concentration of 0.5M was thicker. At a concentration of 1 M, the first reduction wave was smaller, but the electrode passivation could be confirmed by the absence of reduction peak during a second potential scanning. The PEG coating was confirmed by IR-RAS with the characteristic peak of the C-O bonds at 1114cm^{-1}.

EXAMPLE 8

Spin-coating of a poly(vinyl chloride) (PVC) top-coating onto PCL- coated steel.

Since poly(vinyl chloride) (PVC) is compatible with PCL, a THF solution of PVC was spin-coated onto poly(ACL) and poly(ACL-co-CL) coatings prepared according to EXAMPLE 4. The PCV top coatings was confirmed by IR- RAS with the bands at 2910 and 1721 cm^{-1} for alkyl and carbonyl groups adsorptions. Peeling tests (ASTM D3330M-90) showed that the PVC coating was adhering strongly to poly(ACL-co-CL) (the PVC film was not removed after peeling with an adhesion strength superior to 3700N/m) but not to poly(ACL) (the PVC film was removed by peeling with an adhesion strength of 100N/m).

EXAMPLE 9

Electrografting of N-acryloyloxysuccinimide with subsequent grafting of polystyrene containing primary amine groups. "Grafting onto" approach. Scheme 6.

a) Grafting of N-acryloylaxy succinimide.

Electrografting has been performed in the conditions described example 1, where ethyl acrylate has been replaced by N-acryloyloxy succinimide (concentration ranging from 0.1-3M) thus a monomer bearing a succinimidyl function as anchoring group). This monomer was prepared by reaction of 10 g of N hydroxysuccinimide (86.9 mmol) with 7.1 ml of acryloyl chloride (86.9 mmol) in 200 ml of dried CH_2Cl_2 in presence of 12.1 ml of triethylamine (86.9 mmol).The reaction is performed overnight at room temperature with a yield after purification of 95%.The monomer structure was confirmed by RMN H (CDCl3): _ (ppm): 2.84 (s, 4H, $C\underline{H_2}C\underline{H_2}$) , 6.14 (d, 1H, $C\underline{H_2}$=CH), 6.26 (q, C<u>H</u>CO) et 6.68 (d, 1H, $C\underline{H_2}$=CH), and IR (KBr): _ (cm): 3083, 3007 (C-H olefiniques), 2955 (C-H aliphatiques), 1809-1777-1732 (C=O), 1405 (C-N) et 1213 (C-O).

According to the peeling tests (ASTM D3330M-90), the adhesion energy of the coating onto steel was higher than 3070 N/m, as compared with 1850 N/m for neat steel. The film thickness of this primer coating is low (l0nm as determined by XPS) and weakly increased with increasing the monomer concentration from 0.1M up to 3M.]

b) Grafting of poly[meta-(isopropyl-2-amino)styrene-co-styrene) on poly(acrylate succinimidel electrodeposited on steel

A random copolymer of styrene and meta- (isopropyl-2-amino)styrene 98.5:1.5 1 (mol/mol) (Mn = 40000) was spin-coated on the steel plate coated with poly(acrylate succinimide). The amino groups bear by the styrene backbone are complementary reactive groups that can react with X, the succinimidyl activated ester. The electrode was then heated at 150°C under vacuum for 24 h. Unreacted polystyrene was removed by Soxhlet extraction in THF for 2 weeks. The FTIR-RAS spectrum of the modified electrode confirmed the deposition of polystyrene (bands at 3086, 3064, 3028, 2927 and 1601, 1487, 1444 and 1001 cm^{-1} typical for styrene unit). Moreover, the grafted polystyrene film was not detached by the peeling test (ASTM D3330M-90).

c) Grafting offerrocene amine on poly(acrylate succinimide) electrodeposited on steel

Ferrocene amine was grafted onto the polyacrylate succinimide (P in Figure 1) by the grafting onto technique (Figure 2 b). For this purpose, the modified carbon or gold plates were immersed during 6 days at room temperature in 5ml of a DMF solution containing 0.1 g of aminoferrocene (synthesized following Van Leusen D. et al, *Organometallics*, 2001, 20, 224-226.) and a catalytic amount of dimethylaminopyridine. After rinsing in DMF and acetonitrile, the binding of the ferrocene to the surface has been evidenced by cyclic voltammetry (Figure 10) which shows the electroactivity of the ferrocene in the anodic potential range for various scanning rates (100, 50 and 20 mV/s).

d) Grafting of biotine-cadaverine on poly(acrylate succinimide) electrodeposited on steel

Biotine-cadaverine (Aldrich) was grafted onto the polyacrylate succinimide (P in Figure 1) by the grafting onto technique (Figure 2 b). For this purpose, the modified platinum, gold or ITO-glass (see below) plates with grafted polyacrylate succinimide were immersed during 4 days at room temperature in 12.5 mg of biotine- cadaverine (N-(5-aminopentyl)biotinamide) (MM 442.50 30 n=2,83.10^{-5} mol) and 2.85 mg of triethylamine (MM 101.19 n=2,83.10^{-5} mol) and catalytic amount of dimethylaminopyridine dissolved in 2.5 ml of dried DMF. After reaction, the substrate is rinsed in DMF and acetonitrile. The efficient binding of the biotine-cadaverine to the device was evidenced by colorimetric test using dimethylaminocynnamaldehyde (following Vanwetswinkel S., et al., *Bioorgan c & Medicinal Chemistry*, 1995, 3 (7), 907-915.). Figure 11 shows the absorbance of the coating revealed by the dye (curve a) in comparison without the dye (curve b). This spectrum was recorded by UV-VIS spectrophotometer (Hitachi U3300) using electrografted transparent substrates consisting of glass coated with a thin conducting layer of indium-tin oxide (ITO-glass).

EXAMPLE 10

Incorporation of a fluorescent molecule, rhodamine-6G, within a PCL film casted on a grafted PEA-PCL coating, and release in aqueous medium

PCL was grafted from a PEA primer coating electrografted onto steel (20 x 10 x 1 mm-plate), as described in EXAMPLE 1. Three ml of a solution of PCL (10 wt%, Mo=5.8 10^3) and rhodamine-6G (11.2 wt%, 336 ug) in chloroform was cast onto the PEA-PCL coated plates, followed by a chloroform solution of PCL alone to cover the previous PCL with Rhodamine top coating. The plates were then immersed into 1 ml of phosphate buffer (0.13 M, pH 7.4) at 37°C. The whole aqueous volume was collected at regular times and replaced with fresh buffer. The concentration of rhodamine-6G in the aliquots was determined by fluorescence spectrometry (excitation wavelength 526 nm, emission wavelength 550 nm). At the end of the release study (6.75 days), the PCL top coating was no longer adhering and had detached from the surface. However, low-angle IR spectroscopy revealed that the surface remained covered with PCL, probably coming from the electrografted PEA-PCL coating. Rhodamine-6G was released regularly from the PCL coating, with a limited burst effect at early times more likely due to the PCL top coating (Fig. 12).